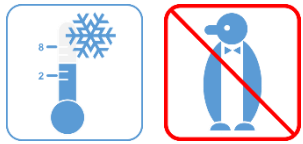
Marvelgent biosciences



Store product at 2°C – 8°C. Do not freeze. The product is shipped at ambient temperature.

ProteIndex™ HIC-Butyl Agarose 4 Fast Flow

ProteIndex™ HIC-Octyl Agarose 4 Fast Flow

ProteIndex™ HIC-Phenyl Agarose 6 Fast Flow, Low Phenyl

ProteIndex™ HIC-Phenyl Agarose 6 Fast Flow, High Phenyl

Intended Use

This product is intended for research use only. Not intended for any animal or human therapeutic or diagnostic use.

Cat. No.	Package Size
11-0240-025	ProteIndex HIC-Butyl Agarose 4 Fast Flow, 25 mL settled resin
11-0240-200	ProteIndex HIC-Butyl Agarose 4 Fast Flow, 200 mL settled resin
11-0242-025	ProteIndex HIC-Octyl Agarose 4 Fast Flow, 25 mL settled resin
11-0242-200	ProteIndex HIC-Octyl Agarose 4 Fast Flow, 200 mL settled resin
11-0244-025	ProteIndex HIC-Phenyl Agarose 6 Fast Flow, Low Phenyl, 25 mL settled resin
11-0244-200	ProteIndex HIC-Phenyl Agarose 6 Fast Flow, Low Phenyl, 200 mL settled resin
11-0246-025	ProteIndex HIC-Phenyl Agarose 6 Fast Flow, High Phenyl, 25 mL settled resin
11-0246-200	ProteIndex HIC-Phenyl Agarose 6 Fast Flow, High Phenyl, 200 mL settled resin

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1. Product Description

ProteIndex™ HIC-Butyl Agarose 4 Fast Flow, HIC-Octyl Agarose 4 Fast Flow, HIC-Phenyl Agarose 6 Fast Flow (Low Phenyl) and HIC-Phenyl Agarose 6 Fast Flow (High Phenyl) are media for Hydrophobic Interaction Chromatography (HIC). They are one of the most frequently used, high throughput techniques for purification of biological macromolecule. The media are well suited for any stage in the purification process from capture to final polish.

The base matrix of these media is highly cross-linked agarose. It provides super physical and chemical stabilities, high-resolution, and high-capacity that meet the needs for biomolecule purification at both laboratory scale and process scale applications. Tables 1 and 2 list the properties of the four media.

Table 1. Characteristics of HIC-Butyl Agarose 4 FF and HIC-Octyl Agarose 4 FF.

	Octyl Agarose 4 FF	Butyl Agarose 4 FF
Matrix	Highly cross-linked 4% agarose	
Type of ligand	Octyl	Butyl
Capacity (/ml medium)	26 mg IgG; 7 mg HSA	7 mg IgG; 26 mg HSA
Particle Size	45 – 165 µm	
Flow rate	≥150 cm/h	
pH stability	3-13	
Storage buffer	20% ethanol	
Storage	2°C – 30°C	

Table 2. Characteristics of HIC-Phenyl Agarose 6 FF, Low Phenyl and high Phenyl.

	Phenyl Agarose 6FF, Low Phenyl	Phenyl Agarose 6FF, High Phenyl
Matrix	Highly cross-linked 6% agarose	
Ligand	~25 µmol phenyl /mL medium	~40 µmol phenyl /mL medium
Capacity (/ml medium)	10 mg IgG; 24 mg HSA	30 mg IgG; 36 mg HSA
Particle Size	45 – 165 µm	
Flow rate	300 – 600 cm/h	
pH stability	3 – 13	
Storage buffer	20% ethanol	
Storage	2°C – 30°C	

2. Characteristics of Hydrophobic Interaction Chromatography Media

HIC-Butyl Agarose 4 Fast Flow is aliphatic hydrophobic interaction medium, which consists of 90µm beads of highly cross-linked agarose. The butyl group is coupled to beads by ether linkage, giving a hydrophobic medium with minimal leakage and no ionic properties.

HIC-Octyl Agarose 4 Fast Flow is aliphatic hydrophobic Interaction medium, which consists of 90µm beads of highly cross-linked agarose. The octyl group is coupled to beads by ether linkage, giving a hydrophobic medium with minimal leakage and no ionic properties.

HIC-Phenyl Agarose 6 Fast Flow, Low Phenyl and **HIC-Phenyl Agarose 6 Fast Flow, High Phenyl** consist of 90 µm beads of 6% highly cross-linked agarose. The phenyl group is

coupled to beads by ether linkage, giving a hydrophobic medium with minimal leakage and no ionic properties. According to the required separation selectivity, efficiency and binding capacity. Different substituted medium are available.

3. Operation

3.1 Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter all buffers by passing through a 0.22 μm or 0.45 μm filter before use.

Binding/Wash Buffer: 0.05 M phosphate, 1.7M $(NH_4)_2SO_4$, pH7.0

Elution Buffer: 0.05 M phosphate, pH7.0

Note: The salt concentration of buffer is high in the Binding/Wash Buffer, and low in the Elution Buffer. The buffers of HIC should be determined empirically according to the samples and the medium being used.

3.2 Sample Preparation

It is recommended to filter the sample solution by passing through a 0.22 μm or 0.45 μm filter before use.

The salt concentration in the sample should be the same as Binding /Wash Buffer. It is usually 0.5 ~ 2.0 M $(NH_4)_2SO_4$.

3.3 Packing Columns

1. Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
2. Close the column outlet leaving the net covered with packing buffer.
3. Resuspend the resins stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

   If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column

to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4. Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, the medium is packed at a constant pressure of approximately 1 bar (0.1 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.
5. When the bed has stabilized, close the bottom outlet and stop the pump.

   If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.
6. With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
7. Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.
8. Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

3.4 Column Packing Evaluation

When column packing is complete, equilibrate the column with up to 5 CV equilibration buffer. To test the effectiveness of column packing, inject a sample of a low molecular weight, unretained compound (for example, acetone or 1M NaCl). If acetone is used as the test marker (use a conductivity monitor), then the equilibration buffer salt concentration should be 100 – 200 mM. The sample volume should be 1 – 3% of the total column volume. Column testing should be operated using the same linear velocity used to load and/or elute the sample.

To obtain comparable height equivalent to a theoretical plate (HETP) values among columns, the same conditions must be applied. Minimum theoretical plate values should be 1,000-3,000 plates/m for linear velocities of 50 – 500 cm/hr.

HETP=L/N

$N=5.54(Ve/W_{1/2h})^2$

L=Bed height (cm)

N= Number of theoretical plates

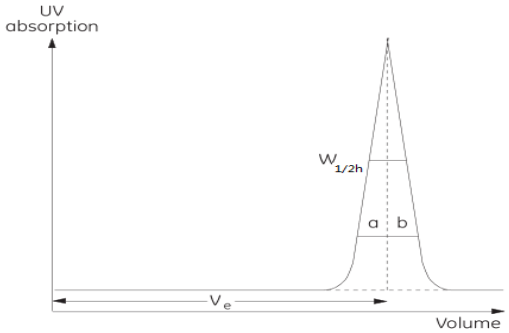
V_e=Peak elution volume or time

$W_{1/2h}$=Peak width at peak half height in volume or time

V_e and $W_{1/2h}$ should always be in the same units

Peaks should be symmetrical and the asymmetry factor as close as possible to 1. Values of 0.8~1.8 are acceptable.

Peak asymmetry factor calculation:
$A_s=b/a$
a = Front section of peak width at 10% of peak height bisected by line denoting V_e
b = Latter section of peak width at 10% of peak height bisected by line denoting V_e
A_s=0.8 - 1.8 is acceptable.



3.5 Sample Purification

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.
2. Wash the column with 10 column volumes of binding buffer.
3. Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

   Note: Hydrophobic interaction is weaker at lower temperatures. This must be taken into account if chromatography is done in a cold room.
4. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
5. Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

3.6 Analysis

Identify the fractions using UV absorbance, SDS-PAGE, or the Western blot.

4. Regeneration

After each run, elute reversibly bound material with low ionic strength buffer. Wash the column with 5 column volumes of distilled H_2O and starting buffer.

5. Cleaning-in-Place

Removing strongly bound hydrophobic proteins, lipoproteins, and lipids

1. Wash the column with 3 column volumes of 70% ethanol or 30% isopropanol (apply increasing concentration gradients to avoid air bubbles formation).

   Alternatively, wash the column with 3 column volumes of 0.1 – 0.5% detergent in a basic or acidic solution. For example, wash with 0.1 – 0.5% non-ionic detergent in 0.1 M acetic acid. Contact time 1 – 2 h.

2. Wash the column with distilled H_2O and re-equilibrate.

Sanitization to minimize microbial contamination

1. Wash the column with 0.5 – 1 M NaOH. Contact time 30 – 60 min.
2. Wash the column with distilled H_2O and re-equilibrate.

6. Storage

Store the HIC-Butyl Agarose 4 Fast Flow, HIC-Octyl Agarose 4 Fast Flow, HIC-Phenyl Agarose 6 Fast Flow (Low Phenyl) and HIC-Phenyl Agarose 6 Fast Flow (High Phenyl) in 20% ethanol at 2 – 30°C.

7. Related Products

Product Name	Package Size	Cat. No.
ProteIndex HIC-Butyl Agarose 4 FF Prepacked Cartridge		
	5x1 mL settled resin	11-0241-5x1
	1x5 mL settled resin	11-0241-1x5
	5x5 mL settled resin	11-0241-5x5
ProteIndex HIC-Octyl Agarose 4 FF Prepacked Cartridge		
	5x1 mL settled resin	11-0243-5x1
	1x5 mL settled resin	11-0243-1x5
	5x5 mL settled resin	11-0243-5x5
ProteIndex HIC-Phenyl Agarose 6 FF Prepacked Cartridge, Low Phenyl		
	5x1 mL settled resin	11-0245-5x1
	1x5 mL settled resin	11-0245-1x5
	5x5 mL settled resin	11-0245-5x5
ProteIndex HIC-Phenyl Agarose 6 FF Prepacked Cartridge, High Phenyl		
	5x1 mL settled resin	11-0247-5x1
	1x5 mL settled resin	11-0247-1x5
	5x5 mL settled resin	11-0247-5x5

Marvelgent Biosciences Inc.
116 Will Dr., Canton, MA 02021, USA
Toll-free: 1.888.330.6623
Email: cust.support@marvelgent.com
www.marvelgent.com